

REPRODUCTIVE BIOLOGY OF THE RARE “COPPER MOSS” *MIELICHHOFERIA MIELICHHOFERIANA*

A. Jonathan Shaw, N. J. Niguidula and T. M. Wilson

Department of Biology

Ithaca College

Ithaca, NY 14850, U.S.A.

INTRODUCTION

The “copper mosses” are unique among land plants. These plants, which actually include both mosses and liverworts, have extremely broad geographic ranges that typically span several continents. Broad intercontinental distributions are not, of course, especially remarkable for bryophytes, as most species of mosses and liverworts have wide ranges compared to seed plants. It is the highly restricted and disjunct occurrences with each site hundreds or even thousands of miles from the nearest station, a general rarity of sexual reproduction over much of their ranges, and at least some association with deposits of copper and/or other metals, that sets the copper mosses apart from other plants. The fact that several species of copper moss often co-occur at precisely the same widely separated stations makes their geographic distributions and ecological characteristics unique and even more bizarre.

There are different kinds of rare plant species, each with different biological causes. Rarity resulting from habitat specificity is one of three basic types recognized by Rabinowitz (1981) and others interested in explaining the biological basis of rarity. These species are thought to be sporadic in occurrence primarily because their habitats are discontinuous and uncommon. Rarity resulting from edaphic specificity has been most thoroughly studied with regard to serpentine endemism (Kruckeberg 1984), but species restricted to deposits enriched with heavy metals such as copper and zinc are also known (Antonovics *et al.* 1971). Some highly restricted taxa are endemic to deposits at one or a few metal mines and appear to have originated *in situ* from widespread, more ecologically generalized species. For example, *Mimulus cupriphilus* Macnair (Macnair 1989) is known only from two copper mines in the foothills of the Sierra Nevada Mountains of California and appears to be a derivative of the widespread species, *Mimulus guttatus* Fischer *ex* DC. Perhaps a comparable example among the mosses is *Ditrichum plumbicola* Crundw., described from lead mine wastes in Great Britain and clearly related to the more widespread species, *D. lineare* Sw. (Crundwell 1976). Species known as copper mosses, however, are very different biogeographically and ecologically from these narrow endemics, and certainly represent a different form of rarity reflecting different ecological limitations and evolutionary histories. The widely distributed, disjunctive copper mosses pose biological questions that are without parallel in any other plant group.

The copper mosses themselves are ecologically and taxonomically heterogeneous. They include about ten species of mosses and liverworts representing eight families. For most species the relationship with copper appears to be facultative, and typical substrate copper levels vary from one species to another (Shaw 1990). *Mielichhoferia mielichhoferiana* (Funck) Loeske was one of the first plants to be

recognized as a copper moss. The species was described on the basis of collections made by Malthias Mielichhofer in 1815 on copper mine tailings in the Salzburg area of what is now Austria, and numerous subsequent authors have reported a correlation between occurrences of *M. mielichhoferiana* and copper deposits (reviews in Persson 1956; Shacklette 1967; Shaw 1990). The genus *Mielichhoferia* is comprised of about 25 species in South America, Africa, and some of the Pacific Islands, and is represented by 3–4 species in North America and northern Eurasia. *Mielichhoferia mielichhoferiana*, with scattered occurrences across North America, Europe, and Asia (south to Japan), is the most widespread of the northern taxa. In contrast, *M. tehamensis* Showers is found only around Mt. Lassen, a volcanic peak in north-central California, and *M. macrocarpa* (Hook. ex Drumm.) Bruch & Schimp. ex Jaeg. is known from about ten disjunct sites in Canada and the United States and at one extremely isolated site in central Asia.

Research described in this paper deals with certain factors that might control the distribution and abundance of *Mielichhoferia mielichhoferiana*. The species is exceedingly rare throughout its range. For example, in the northeastern United States, it has been found at only one site each in Maine and Michigan, and at two sites separated by over 300 km in New York. From these sites in the northeastern and north-central United States, the species is disjunct at about five proximate localities (i.e., within a few km) in the southern Appalachian Mountains along the border of Tennessee and North Carolina (Schofield 1959). Additional disjunct populations occur in the western United States, boreal and arctic Canada, and Alaska.

It has not been demonstrated that the presence of higher than normal concentrations of copper is the critical factor limiting the abundance of *M. mielichhoferiana* (or, in fact, any other copper moss). Although substrate copper concentrations reported in the literature for this species tend to be elevated relative to most soils (Persson 1956; Shacklette 1967; Shaw 1990), a considerable number of populations grow on soils or rocks with only trace amounts of copper (Shaw 1990, unpublished data). The species may show an association with sulfur (Schatz 1955) rather than copper (most copper deposits also contain high sulfur), but little data on substrate sulfur concentrations are available. Substrates of *M. mielichhoferiana* are exceptionally acidic and this ecological characteristic is in fact more consistent across the range of the species than is enrichment with copper: 21 North American soils samples had the remarkably low mean pH of 3.6 (unpublished data). Experimental studies of mineral nutrition in *M. mielichhoferiana* will be reported in a later paper.

This paper reports studies on the reproductive biology of *M. mielichhoferiana*. Previous discussions of factors limiting the distribution and abundance of copper mosses have focussed on substrate chemistry, but the rarity of spore formation suggests that these species may have limited potential for dispersal and establishment of plants in nature. Virtually nothing is known about reproductive processes in natural populations of the copper mosses. It has been suggested that plants rare because of extreme habitat specificity might be genetically uniform (Huenneke 1991). This uniformity could result from founder events and population bottlenecks that occur during colonization of disjunct localities, and inbreeding due to small population sizes. This might be especially true of the copper mosses such as *M. mielichhoferiana*, in which sexual reproduction appears to occur infrequently. A series of questions were addressed in this study. What factor(s) limit sporophyte formation in natural populations? When sporophytes are formed, do they contain viable spores? How do germination percentages vary among sporophytes and populations? Do germinating spores produce viable gametophytes? Do populations dif-

fer in gametophytic growth rates? Are gametophytic plants capable of vegetative regeneration following prolonged dormancy and does this capability vary among populations?

MATERIALS AND METHODS

Plants from widely disjunct populations were included in the experimental work (Fig. 1). These five populations represent five states spanning a range from the Atlantic to the Pacific coasts of the United States. In **New York**, *M. mielichhoferiana* grows on moist, somewhat crumbly metamorphic rock along Cattaraugus Creek and several of its tributaries in Cattaraugus and Erie Co. (no. 1 in Fig. 1). The species occurs at several localities within a few kilometers of each other. Copper occurs at trace concentrations only at these sites. Collections of *M. mielichhoferiana* were originally made from Cattaraugus Creek in 1941. It presently



FIG. 1. North American map showing the distribution of collections used in the experimental studies on *Mielichhoferia mielichhoferiana* described in this paper.

occurs as numerous colonies of varying sizes on vertical or nearly vertical rock. The only **Michigan** population of *M. mielichhoferiana* (FIG. 1, no. 2) grows on moist rocks along the south shore of Lake Superior in Pictured Rocks National Lakeshore. Professor G. E. Nichols collected *M. mielichhoferiana* there originally in August, 1933, the first record for this species in North America (Andrews 1933). Numerous collections of *M. mielichhoferiana* have been made subsequently from Pictured Rocks, but the population has never been marked and it is not clear to what extent all collections derive from precisely the same population. The present collection, made during October, 1990, originated at a north-facing site subject to harsh winds and severe weather from Lake Superior. Field work at several of the other accessible areas along Lake Superior did not reveal additional sites for the species. Shacklette (1967) reported elevated copper levels in substrates of *M. mielichhoferiana* from Pictured Rocks. In fact, the Upper Peninsula of Michigan is famous for extensive copper deposits that have been mined for hundreds of years.

In **Tennessee** (FIG. 1, no. 3; actually on the North Carolina border), *M. mielichhoferiana* occurs at several sites within a few kilometers of each other in the Great Smoky Mountain National Park (Schofield 1959). The experimental population grows at "Charlie's Bunyan", a large outcrop of schist amidst a mixed hardwood-conifer forest at approximately 5000 ft. elevation. The moss is abundant in protected recesses on the rock.

In **Colorado**, *M. mielichhoferiana* occurs at scattered localities and has been found at a number of abandoned mines in Ouray Co. At one mine, *M. mielichhoferiana* occurs with *M. macrocarpa*. The site is one of only three areas that *M. macrocarpa* has been found in the United States outside of Alaska. The experimental population grew on mine tailings at approximately 10,000 ft elevation just north of the summit of Red Mountain Pass (FIG. 1, no. 4). The last experimental population, from **California** (FIG. 1, no. 5), occurred on decomposing metamorphic rock along a roadside in the Sierra Nevada Mountains. The moss is abundant at the site, but only over a distance of about 25 meters along the road. Field work did not reveal any additional sites in the area.

The reproductive behavior of gametophytic plants was investigated by intensively sampling individuals from the New York population. Preliminary work showed that plants form gametangia in the spring and sporophytes mature in late summer and autumn. During June of 1990, 317 plants were collected and scored for the presence of archegonia or antheridia. Plants occurred on the gorge walls as discrete colonies that were sampled independently in order to assess small scale between-colony differences in reproductive behavior. This sampling scheme was repeated in 1991, when a total of 419 plants were collected. During the second year of sampling, the sizes of individual colonies were estimated in order to determine if there was a relationship between colony size and sexual expression. For each colony, the longest two dimensions were measured in centimeters.

Spore germination percentages were estimated for 30 sporophytes from the Michigan population, and ten each from the New York and California populations in the following manner. Sporophytes were surface sterilized by soaking in full strength bleach for 8 minutes, rinsed in sterile distilled water, and the spore contents were emptied into 4 ml of distilled water. 0.5 ml of the spore suspension was then pipetted onto a Knopp's nutrient medium (recipe in Basile and Basile 1988) solidified with 1.2% agar. Three replicate cultures were initiated from each sporophyte and the petri dishes were completely randomized on a lighted shelf with a 14 hour photoperiod.

Another experiment was initiated to determine if germinating spores produce vigorous sporplings, and whether sporpling vigor varies among natural populations. Spores from five sporophytes each from the Michigan, New York, Tennessee, and California populations were inoculated on Knopp's medium. Only one sporophyte was included from the Colorado population because no others were available. Two days after the spores germinated, the lengths of ten randomly selected sporplings were measured under a compound microscope. Three replicate cultures of each sporophyte were inoculated and the dishes were completely randomized as in the previous experiment.

The viability of gametophytes following a prolonged period of dormancy was assessed by incubating stem fragments in liquid Knopp's medium. Gametophytic plants were collected from a series of Colorado populations during the summer of 1990. These plants were then stored as herbarium specimens until the spring of 1991. In April, 1991, 25 stems from each population were placed in Knopp's medium in 5 ml sterile tissue culture tubes (one stem per tube). After one month, the presence or absence of renewed gametophytic growth was scored in each tube. This growth was most commonly in the form of elongation and branching of the original fragment. In some cases, protonemata and/or rhizoids were also produced. A second and third replicate block of 25 stems from each population were initiated during the spring and summer of 1991, and each was scored in a similar manner following one month of incubation in Knopp's medium.

RESULTS AND DISCUSSION

Few sporophytes were observed in the New York population during the 1990 and 1991 seasons. This is consistent with observations made on populations elsewhere in the United States during the last three years in which relatively few sporophytes were observed (unpublished data), and with literature reports in which absence or scarcity of sporophytes has been noted (e.g., Hartman 1969; Schofield 1959). Repeated visits to a population in Colorado showed that successful sporophyte formation varies tremendously from year to year (Hartman 1969). The population at Pictured Rocks, Michigan, on the other hand, has been visited repeatedly since it was discovered in 1933, and numerous herbarium specimens have been collected with abundant sporophytes over the years. Without yearly visits and systematic sampling of populations, however, it is impossible to determine what factors limit sporophyte formation during any given year.

At the New York site, over 90% of the plants collected during both the 1990 and 1991 seasons lacked gametangia of either sex (TABLE 1). In 1990, only six out of 317 plants bore antheridia, and only 20 bore archegonia. In 1991, the percentage of sterile plants was exactly the same as in the previous year (92%), but the proportions of plants bearing archegonia versus antheridia was reversed (TABLE 1). Since the combined abundance of reproductive plants was so low in both years, the difference between male and female reproductive expression in 1990 and 1991 may well be the result of sampling error. On the other hand, the scarcity of reproductive plants in general is clearly at least a proximate cause for the rarity of sporophytes in this population.

Successful sporophyte formation is dependent not only on the formation of archegonia and antheridia, but on their proximity, since gamete dispersal is limited by the ability of free-swimming sperm to reach eggs. Plants of *M. mielichhoferiana*

TABLE 1. Sexual expression in gametophytes from the Cattaraugus Co., New York population of *Mielichhoferia mielichhoferiana*.

	1990	1991
Total Plants	317	419
Male Plants	6 (2%)	27 (6%)
Female Plants	20 (6%)	7 (2%)
Sterile Plants	291 (92%)	385 (92%)
Total Colonies	18	45
Male Colonies	4 (22%)	12 (27%)
Female Colonies	4 (22%)	0
Bisexual Colonies	1 (6%)	1 (2.2%)
Sterile Colonies	9 (50%)	32 (71%)

at the New York site occur in more or less discrete colonies, and when these are separated by more than a few centimeters it is likely that successful fertilization will occur primarily or exclusively between plants within individual colonies. During both 1990 and 1991, only one colony each year was observed with both male and female reproductive plants (TABLE 1). It is not surprising that these colonies were the only ones that bore sporophytes each year. Approximately one fourth of the colonies sampled each year bore male but no female gametangia. During 1990, four colonies contained female but no male gametangia, but in 1991 no such exclusively female colonies were sampled (TABLE 1). The total number of fertile colonies was too low to determine if there was a relationship between colony size and sexual expression. In 1990, the only colony with both males and females was among the smallest ($< 25 \text{ cm}^2$). In 1991, the bisexual colony was by far the largest, occupying several meters² of the rock wall.

Studies of the factors limiting sporophyte formation in moss populations have shown that plants bearing antheridia are often scarce in spite of an abundance of plants with archegonia (review in Longton & Schuster 1983). In some dioicous mosses, spatial separation of male and female plants may severely limit fertilization frequency. At the extreme, male and female plants of some mosses appear to be separated by hundreds or even thousands of miles. For example, North American populations of *Tortula pagorum* contain only female plants, European populations appear to be exclusively male, and sporophytes have been observed only in Australia where both males and females occur (Anderson 1943, Stone 1971). In the New York population of *M. mielichhoferiana*, successful sexual reproduction appears to be limited by the infrequent expression of sexuality in spite of the occurrence of both male and female plants.

The percentage of spores that germinate varied greatly among sporophytes both within and between the three populations for which data are available (Fig. 2). On average, sporophytes from the California population had the lowest germination (mean $42.5\% \pm 11.2 \text{ SE}$) while the two others had almost identical, higher average percentages (New York, $64.9 \pm 9.1\%$; Michigan, $64.8 \pm 5.7\%$). All three populations contained individual sporophytes with very low or zero germination. In fact, about 30% of the sporophytes from the California and Michigan populations contained spore progeny exhibiting lower than 5% germination. Nevertheless, a

PICTURED ROCKS, MI

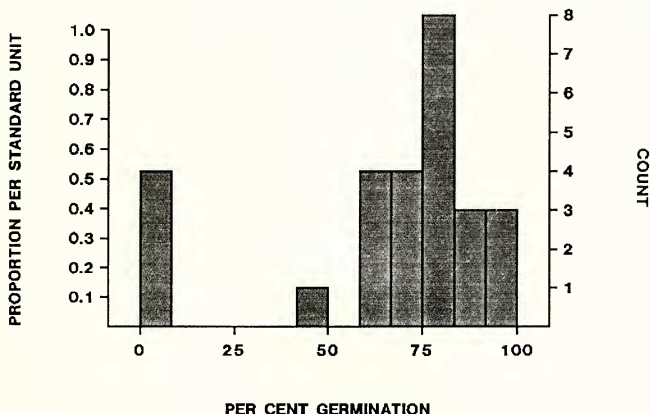


FIG. 2. Germination percentages in families from the Michigan population of *M. mielichhoferiana*. Family means are based on three replicate petri dishes; 35 spores were scored in each dish. The distribution of germination percentages in the California and New York populations were similar, and are not shown. See text for additional discussion.

huge number of viable spores were produced in each of these populations, and numerous studies of other mosses, both rare and common, have shown similar, highly variable germination percentages.

Differences in spore viability between sporophytes may have both genetic and nongenetic determinants. Although "common garden" experiments, including the present study of germination rates, suggest genetic differences, strong environmental effects are also possible, if not likely. In an analogous situation, for example, it has been demonstrated that gametophytic (pollen) vigor can be affected by the nutrient status of the parental sporophyte (Young & Stanton 1990).

Statistically significant differences in sporeling vigor also occurred among families within populations, and among the populations (TABLE 2). On average, plants from the Colorado and Michigan populations grew three times as large as those from California and New York over the same time interval. Tennessee plants were almost exactly intermediate between these two extremes (TABLE 2). The presence of significant variation in vigor between families of gametophytic plants derived from different sporophytes adds support to the interpretation that family differences have a genetic component. Nevertheless, effects of parental sporophyte nutrition and health on progeny performance is still possible, especially as sporeling growth was measured just 48 hours subsequent to germination. The early growth of sporelings may represent what is essentially an extended germination phase, affected by resources obtained by the spore while still in the sporangium.

TABLE 2. Early sporcling growth in five populations of *Mielichhoferia mielichhoferiana* on Knopp's medium. Mean values (\pm standard errors) are based on five families (capsules) per population. Spores from each family were inoculated on three replicate petri plates and 10 sporclings were measured on each plate. The analysis of variance for these data is shown below.

POPULATION	SPORELING LENGTH (μ m)			
California	21.9 \pm 1.4			
Colorado	60.6 \pm 3.5			
Michigan	62.9 \pm 5.3			
New York	20.3 \pm 2.3			
Tennessee	42.6 \pm 8.5			
SOURCE	d.f.	SS	MS	F
Populations	3	2416.5	805.5	16.43**
Families (within pops.)	10	1372.1	137.2	2.80*
Error	27	5130.0	190.0	

* = $P \leq 0.01$; ** = $P \leq 0.0001$

TABLE 3. Gametophytic regeneration following 9–14 months of dormancy in Colorado populations of *M. mielichhoferiana*. Each block consisted of 25 gametophytic fragments per population. Integers represent percentages of growing stems out of the 25 for each population.

POPULATION	BLOCK 1	BLOCK 2	BLOCK 3
Eureka Mine	52	44	4
Gothic	55	44	13
Red Mountain Pass South	40	40	8
Red Mountain Pass North	33	40	8
Telluride	16	28	21
Yankee Boy Basin	85	72	25

Differences between the populations were also evident in studies of gametophytic regeneration following nine to fourteen months of dormancy (TABLE 3). When the first block of fragments was initiated the plants had been dried for approximately 9 months, the second block was initiated when the plants had been dried for 12 months, and the last block when the plants had been dried for 14 months. It is clear that plants from all populations lost viability during this period. Indeed, the decline in regeneration was drastic between 12 and 14 months of dormancy. Nevertheless, some consistent population differences were evident. Plants from Yankee Boy Basin, for example, showed the highest percentage regeneration in every experimental block. Conversely, the Telluride population showed the lowest regeneration during both of the first two blocks, improving relative to others in the third block because viability in other populations had declined so greatly. It is noteworthy that the two populations from Red Mountain Pass responded very similarly in every block.

As with the previous experiments, caution should be exercised in assuming that population differences in regeneration result solely from genetic differentiation rather than environmental effects on plants while they were growing in nature. Nevertheless, consistent population differences are definitely suggestive of genetic variance for tolerance of prolonged desiccation and the ability to resume active

growth upon hydration. Similarity between the two Red Mountain Pass populations, in contrast, may reflect underlying genetic similarity. These two populations originated at abandoned copper mines within a few kilometers of each other. The experimental set-up employed in this study is obviously quite unlike conditions in nature, yet variability revealed by this approach may suggest analogous variation in the propensity for asexual dispersal and regeneration under field conditions.

CONCLUSIONS

Understanding the reasons for rarity is fundamental to conservation science and constitutes one of the most fascinating problems in organismic biology. Prerequisite to understanding why some species of plants are rare is the recognition that there are different kinds of rarity (Rabinowitz 1981). The copper mosses constitute a class of plants that have no counterpart among other organisms. They have the odd combination of being extremely widespread but with highly disjunctive and sporadic occurrences.

The copper moss designation for these species is a misnomer not only because some of the species are liverworts and not mosses, but also because their association with copper is inconsistent and sometimes contradictory. A dependence on elevated copper concentrations in the soil is insufficient to explain the rarity of these species. This study assesses the ability of one of the copper mosses, *Mielichhoferia mielichhoferiana*, to reproduce and therefore maintain viable populations, by determining the factors that limit sexual reproduction and by evaluating the viability and vigor of spores and sporelings. In the New York population of *M. mielichhoferiana*, sporophyte formation appears to be limited by the formation of gametangia. However, those spores that are produced are largely viable, and produce relatively vigorous offspring that are capable of sustained gametophytic growth. Although gametophytic growth was measured in this study just days subsequent to germination, these plants have now formed extensive protonema and gametophores in our laboratory.

Population comparisons show that viable spores are the rule and not the exception, and that a significant amount of phenotypic variability occurs within the species. This variability is manifest as both gametophytic growth and the ability to regenerate after prolonged dormancy, and almost certainly reflects some degree of underlying genetic variability. These observations do not suggest that *M. mielichhoferiana* as a species is genetically depauperate. Nevertheless, small populations of limited size increase the risk that chance events combined with habitat destruction pose a significant risk to the long term survival of this rare species.

ACKNOWLEDGEMENT

Funding for this research was provided by NSF grant no. BSR-9020126.

LITERATURE CITED

- Andrews, A. L. 1933. The *Mielichhoferia* of northern North America. *Bryologist* 35: 38-41.
Anderson, L. E. 1943. The distribution of *Tortula pagorum* (Milde) De Not. in North America. *Bryologist* 46: 47-66.
Antonovics, J., A. D. Bradshaw and R. G. Turner. 1971. Heavy metal tolerance in plants. *Adv. Ecol. Res.* 7: 1-85.

- Basile, D. V. and M. R. Basile. 1988. Procedures used for the axenic culture and experimental treatment of bryophytes. Pp. 1–16 in Gilime, J. M. (ed.), *Methods in bryology*. Proc. Bryol. Meth. Workshop, Mainz.
- Crundwell, A. C. 1976. *Dirichium plumbicola*, a new species from lead-mine waste. J. Bryol. 9: 167–169.
- Hartman, E. L. 1969. The ecology of the "copper moss" *Mielichhoferia mielichhoferi* in Colorado. Bryologist 72: 56–69.
- Huenneke, L. E. 1991. Ecological implications of genetic variation in plant populations. Pp. 31–44 in Falk, D. A. and K. E. Holinger (eds.), *Genetics and conservation of rare plants*. Oxford Univ. Press, New York & Oxford.
- Kruckeberg, A. R. 1984. *California serpentines*. Univ. Calif. Press, Berkeley.
- Longton, R. E. and R. M. Schuster. 1983. Reproductive ecology. In Schuster, R. M. (ed.), *New manual of bryology* I: 386–462. Hattori Botanical Laboratory, Nichinan.
- Macnair, M. R. 1989. A new species of *Mimulus* endemic to copper mines in California. Bot. J. Linn. Soc. 100: 1–14.
- Persson, H. 1956. Studies in "copper mosses". J. Hattori Bot. Lab. 17: 1–18.
- Rabinowitz, D. 1981. Seven forms of rarity. Pp. 205–218 in H. Synge (ed.), *The biological aspects of rare plant conservation*. Wiley, New York.
- Schatz, A. 1955. Speculations on the ecology and photosynthesis of the "copper mosses." Bryologist 58: 113–120.
- Schofield, W. B. 1959. *Mielichhoferia mielichhoferiana* in the Southern Appalachians. Bryologist 62: 248–250.
- Shacklette, H. T. 1967. Copper mosses as indicators of metal concentration. Geol. Surv. Bull. 1198G: 1–18.
- Shaw, A. J. 1990. Metal tolerance in bryophytes. Pp. 133–152 in Shaw, A. J. (ed.), *Heavy metal tolerance in plants: evolutionary aspects*. CRC Press, Boca Raton.
- Stone, I. G. 1971. The sporophyte of *Tortula pagorum* (Milde) De Not. Trans. Brit. Bryol. Soc. 6: 270–277.
- Young, H. J. and M. L. Stanton. 1990. Influence of environmental quality on pollen competitive ability in wild radish. Science 248: 1631–1633.